

## Review Article

# Nutritional modulation of vascular function

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**ABSTRACT:** *Inflammation and oxidative stress are important contributors to atherosclerosis initiation, progression, and clinical complications. The inflammatory process, which is promoted by several cardiovascular risk factors, involves both a local microinflammatory response and a systemic low-grade inflammation. Oxidative stress significantly contributes to vascular damage, amplifies the inflammatory response, and is responsible for low-density lipoprotein (LDL) oxidation, which triggers plaque formation. Several nutritional strategies that can impact inflammation and oxidative stress have been identified. Omega-3 fatty acids, antioxidant vitamins, carotenoids, and polyphenols may all influence atherosclerosis initiation and clinical progression. Large-scale clinical trials have been conducted to assess the effects of some of these nutritional principles on selected cardiovascular end points. Optimization of nitric oxide bioavailability to the vessel wall by nutritional modulation of its production may represent a winning strategy in primary and secondary prevention of cardiovascular disease. Based on clinical evidence, routine antioxidant supplementation is currently not recommended in the general population for the primary and secondary prevention of cardiovascular disease. Instead the benefits of a diet rich in fruit and vegetables should be emphasized in nutritional counseling. (Nutritional Therapy & Metabolism 2008; 26: 49-58)*

**KEY WORDS:** *Antioxidants, Atherosclerosis, Inflammation, Oxidative stress, Nitric oxide, Nutrition*

## INTRODUCTION

Cardiovascular disease is an important contributor to morbidity and mortality in the general population. Elucidation of the pathophysiologic mechanisms initiating the atherosclerotic process and promoting its progression and acute complications has definitely allowed us to define nutritional interventions to prevent and/or retard the atherosclerotic process.

A number of studies have demonstrated that inflammation represents the key mechanism promoting atherosclerosis (1, 2). Cardiovascular risk factors activate the endothelial cells, resulting in the production of adhesion molecules (E-selectin, vascular cell adhesion molecule-1 [VCAM-1], and intercellular adhesion molecule-1 [ICAM-1]) (3). Receptors for adhesion molecules are located on monocytes and lymphocytes, which adhere to the endothelium and migrate to the subendothelial matrix (4). Monocytes then differentiate to macrophages under the stimulation of macrophage colony-stimulating factor, produced by the intima (5). Activated macrophages express a number of receptors, including the toll-like receptors and scavenger receptors for oxidized low-density lipoproteins (LDLs), endotoxins, and apoptotic

bodies (6). Once phagocytosed these products stimulate the differentiation of macrophages in foam cells. Removal of oxidized LDLs is an important part of the protective role of macrophages in the inflammatory response (7) to protect endothelial and smooth muscle cells from the deleterious effects of modified LDLs. Dietary antioxidants (especially vitamin E) protect LDL from oxidation and thus may prevent or delay the atherosclerotic process. The toll-like receptors, upon binding with bacterial toxins, heat shock proteins, and stress proteins, initiate signaling cascades that result in the production of proinflammatory cytokines and oxygen-derived free radicals (6).

The activation of T cells, the activation of the complement cascade, and the production of autoantibodies are also important players in atherosclerosis initiation. Atherosclerotic plaques are characterized by a T-cell infiltrate (8). Activated T lymphocytes can produce proinflammatory cytokines such as interferon- $\gamma$  and tumour necrosis factor beta (TNF $\beta$ ) which can stimulate macrophages, endothelial cells, and vascular smooth muscle cells (9). Activated endothelial cells produce interleukin-6 (IL-6). By acting synergistically, these cytokines stimulate the production of inflamma-

tory and cytotoxic mediators by macrophages, further amplifying the proinflammatory response. As a result the systemic circulating levels of IL-1 and IL-6 can increase and stimulate the hepatic production of C-reactive protein (CRP), promoting a systemic inflammatory response. The local release of proinflammatory cytokines (interleukins and TNF $\alpha$ ) activates polymorphonucleates which adhere and migrate through the endothelial layer. They release growth factors which stimulate the replication of vascular smooth muscle cells and the formation of a thick extracellular matrix which characterizes the most advanced atherosclerotic lesions. In addition, activated leukocytes release reactive oxygen species, including hydrogen peroxide, nitric oxide, and peroxynitrite which further damage the endothelial cells (10).

Inflammatory processes not only stimulate the initiation and evolution of atherosclerotic plaques, but can also accelerate the acute thrombotic complications (2). Activated macrophages and T lymphocytes produce proinflammatory cytokines, proteases, coagulation factors, reactive oxygen species, and other vasoactive molecules which contribute to plaque instability. Cumulatively, these factors inhibit the formation of a fibrous cap, attack collagen, and begin thrombus formation (11). Plaque ulceration results in thrombosis and eventually in ischemic embolic complications far from the site of origin. All of these reactions can induce plaque activation and rupture, thrombosis, and ischemia. The importance of activation of the inflammatory process in the pathogenesis of atherosclerosis progression and complications is strengthened by the observation that a number of clinical studies have shown a direct association between circulating levels of acute phase reactants (especially CRP), cardiovascular risk, and clinical outcome in the general population (12-14).

In addition, another important contributor to the atherosclerotic process is oxidative stress. All cells of the vessel wall produce reactive oxygen species, which physiologically regulate a number of functions, including cell proliferation, apoptosis, inflammation, and collagen production. The production of reactive oxygen species is increased in atherosclerosis (15), overwhelming the plasma antioxidant capacity. Oxidative stress directly results in vascular damage. Plasma markers of oxidative stress strictly and directly correlate with cardiovascular complications in patients with stable moderate coronary artery disease (16). Oxidative stress is also implicated in the amplification of the inflammatory response by inducing the pivotal molecule of the inflammatory response, nuclear factor kappa beta (17), which induces the production of cytokines and adhesion molecules. Therefore, nutritional strategies to

reduce oxidative stress and/or increase antioxidant capacity, may be helpful in preventing and/or retarding vascular injury in atherosclerosis. The available nutritional strategies to modulate inflammation and oxidative stress to reduce vascular damage will be discussed.

## NUTRITIONAL INTERVENTIONS TO MODULATE VASCULAR DISEASE

Nutritional interventions can beneficially impact atherosclerotic disease either by blunting vascular inflammation and/or preventing LDL oxidation. Another important nutritional approach is focused on restoring the functionality of a key enzyme in endothelial function, the nitric oxide synthase enzyme.

### Antiinflammatory strategies

Omega-3 fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are well represented in fish, are the major products of  $\alpha$ -linolenic acid. A number of epidemiologic studies have established an inverse association between fish consumption and cardiovascular morbidity and mortality (18-22). Secondary prevention interventional studies have confirmed that supplementation with omega-3 fatty acids reduces mortality and the number of acute cardiovascular events in high-risk patients (23, 24). The underlying mechanisms by which these beneficial effects are achieved include a significant reduction of plasma lipids, blood pressure, platelet aggregability, and number of arrhythmias (25).

However, there is increasing evidence that an important mechanism by which omega-3 fatty acids prevent the progression of atherosclerosis is their antiinflammatory activity. In fact, plasma levels of omega-3 fatty acids are inversely associated with circulating proinflammatory cytokines (IL-6, TNF $\alpha$ , and CRP) and directly with antiinflammatory markers (IL-10 and TGF $\alpha$ ) (26-28). In addition, omega-3 fatty acids (especially DHA) down-regulate the expression of adhesion molecules in the endothelium, resulting in blunted leukocyte rolling and adhesion, and stimulate the production of nitric oxide from endothelial cells; this effect in vivo translates into ameliorated endothelial function (29). Omega-3 fatty acids also down-regulate the expression of cyclooxygenase-2, an important proinflammatory protein which plays a role in plaque activation and rupture (30). Finally, incorporation of omega-3 fatty acids into cell membranes results in a decrease in the omega-6 to omega-3 fatty acid ratio. It is well known that endothelial activation is stimulated by omega-6-

derived eicosanoids, which are characterized by a marked proinflammatory and prothrombotic activity compared with omega-3–derived eicosanoids (31).

Higher dietary fiber intake is associated with reduced cardiovascular risk (32, 33). This beneficial effect is achieved by a constellation of metabolic actions exerted by soluble (i.e., pectin and beta-glucans) and insoluble fibers: the former decrease plasma LDL cholesterol and insulin resistance, the latter improve blood pressure and reduce plasma apolipoprotein B concentrations, triacylglycerols, and homocysteine. In addition, recent clinical data have shown that dietary fiber consumption is associated with significantly reduced plasma concentrations of inflammatory markers (34, 35). These findings contribute to further explain the beneficial effect of dietary fibers on cardiovascular risk, which may be mediated by a down-regulation of systemic low-grade inflammation that characterizes and promotes atherosclerosis initiation, progression, and clinical complications. Consumption of mixed different types of fibers deriving from fruit, vegetables, and whole grain is highly recommended in clinical practice for the prevention of cardiovascular disease (36).

### Nutritional modulation of LDL oxidation

Several bodies of evidence support the hypothesis that modulation of oxidative stress has beneficial effects in atherosclerosis. Epidemiologic studies have demonstrated that reduced levels of plasma antioxidants are associated with cardiovascular disease and that enhanced intake of these compounds is protective against atherosclerosis. Vitamin E is a lipid-soluble antioxidant which prevents LDL oxidation. Its effects have been the object of a number of population studies targeted at cardiovascular disease.

The term *vitamin E* includes a group of different natural compounds (tocopherols and tocotrienols) among which  $\alpha$ -tocopherol demonstrates the highest antioxidant capacity (37). In addition, synthetic forms of vitamin E are available, which differ from vitamin E for antioxidant capacity, bioavailability, elimination, and tissue uptake. Once ingested, vitamin E is incorporated into biological membranes of plasma lipoproteins. Within them, vitamin E protects polyunsaturated fatty acids from oxidation. A number of other antioxidant compounds are involved in regenerating oxidized vitamin E, and they include the water-soluble antioxidant vitamin C and the lipid-soluble antioxidant coenzyme Q10.

A number of studies have confirmed the free radical scavenger effect of vitamin E preventing LDL oxidation in vitro and in vivo (38, 39) and restoring endothelial function (40, 41). Recently it has become clear that vita-

min E exerts other potentially antiatherosclerotic effects, which are reflected in the inhibition of protein kinase C, preventing vascular smooth muscle cell proliferation (42), platelet adhesion and aggregability (43), plasma thrombin production (44), superoxide anion production by monocytes (45), and finally reduction of monocyte adhesion to endothelial cells, blocking the expression of adhesion molecules (46). In endothelial cells, vitamin E stimulates the production of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation (47), and improves endothelial function (48). Interestingly, it has been reported that the antiinflammatory effects characterize only natural and not synthetic forms of vitamin E (49) and that the antioxidant effects of vitamin E on LDL oxidation are dose-dependent (50).

Many observational studies have assessed the relationship between dietary vitamin E intake and cardiovascular disease. The US Nurses' Health Study and the Health Professional's Follow-up Study demonstrated a significant risk reduction (by 41% and 37%, respectively) in patients taking vitamin E supplementation (51, 52); these results were confirmed also by the Established Populations for Epidemiologic Studies of the Elderly (EPESE) (53), while the Iowa Women's Health Study failed to demonstrate any effect of vitamin E supplementation on cardiovascular risk (54). Despite these findings, the data obtained by large primary and secondary prevention interventional studies on cardiovascular end points have yielded conflicting results (Tab. I). A number of possible reasons have been hypothesized, such as the different doses and combinations of antioxidants used ( $\alpha$ -tocopherol alone or in combination with ascorbic acid), the degree of oxidative status in the study population, and the selected clinical end points. However, it seems that natural vitamin E is more effective than synthetic vitamin E probably because of adjunctive antiinflammatory effects and that a daily dose > 400 IU is crucial (50). Finally, selection of the most efficient combination of antioxidants has important effects on clinical results. Combination of vitamin E with beta carotene did not result in reduced cardiovascular mortality (64), and beta carotene alone has been shown to have a prooxidant activity in selected clinical settings (65). Instead a combination of vitamin E with ascorbic acid seems to be the most efficient in regenerating oxidized  $\alpha$ -tocopherol and preventing its prooxidant effects at higher concentrations (66).

It is known that a diet rich in fruit and vegetables is inversely associated with cardiovascular risk, which decreases progressively with increasing daily servings of vegetables (67). Carotenoids are a class of pigments contained in the skin of fruit and berries conferring on them their characteristic pigmentation. To date 34

TABLE I - ANTIOXIDANT RANDOMIZED CLINICAL TRIALS

Study	Subjects	Dosage	Duration (years)	Results
Primary prevention				
VEAPS (55)	322	400 IU/day synthetic $\alpha$ -tocopherol	3	No effect on carotid atherosclerosis regression
ATBC (56)	27,271	Synthetic vitamin E 50 mg/day, $\beta$ -carotene 20 mg/day, alone or in combination or placebo	6.1	Fatal MI: RR 0.92 (95% CI, 0.81-1.05). Primary combined end point (nonfatal MI and cardiovascular death): RR 0.96 (95% CI, 0.88-1.04); total mortality: -2%, nonsignificant
ASAP (57)	440	$\alpha$ -Tocopherol 272 IU/day and vitamin C 500 mg/day	6	Significant reduction of carotid atherosclerosis progression
PPP (58)	4,495	Synthetic vitamin E 330 mg/day vs. aspirin 100 mg/day	3.6	No effects of vitamin E on cardiovascular mortality and MI
Secondary prevention				
CHAOS (59)	2,002	$\alpha$ -Tocopherol 800 or 400 IU/day or placebo	1.5	Primary end point: Nonfatal MI, RR 0.53 (95% CI, 0.34-0.83), $p = 0.005$ . Nonsignificant increase in cardiovascular mortality
GISSI (60)	11,324	Synthetic vitamin E 330 IU/day	3.5	No effect on primary end point (death, nonfatal MI, and stroke); secondary end point: cardiovascular death: RR 0.80 (95% CI, 0.65-0.99), -4.7%, nonsignificant
SPACE (61)	196	$\alpha$ -Tocopherol 800 IU/day	519 days	Significant reduction in primary combined end point (MI fatal and nonfatal, ischemic stroke, peripheral vascular disease, and unstable angina). No effect on secondary end points (total mortality and cardiovascular disease mortality)
HPS (62)	20,536	Synthetic vitamin E 600 mg/day, vitamin C 250 mg/day, $\beta$ -carotene 20 mg/day	5	No effects on cardiovascular mortality and MI
HOPE (63)	9,541	$\alpha$ -Tocopherol 400 IU/day or placebo	4.5	No effects on MI, stroke, and cardiovascular mortality

MI = myocardial infarction; RR = relative risk; 95% CI = 95% confidence interval.

carotenoids have been characterized in human serum, differing for antioxidant activity (which is highest in astaxanthin) (68) and provitamin A activity ( $\beta$ -carotene) (69). Beta carotene retards LDL oxidation in vitro (70), and thus it has been intensively studied for its possible use in the prevention of cardiovascular complications. Available evidence shows that beta carotene, alone or in combination with vitamin E, has no effect on atherosclerosis progression or paradoxically is associated with increased cardiovascular events both in primary and secondary prevention trials (56, 62, 71). Thus carotenoids other than beta carotene may be important in explaining the beneficial effects of fruit and vegetables on cardiovascular risk. Lycopene is a carotenoid especially contained in tomatoes (72). Several studies have shown an inverse association between lycopene assumption and prostate cancer. In addition, as demonstrated by clinical trials, lycopene concentrations, especially in adipose tissue, are inversely associated with

the risk of myocardial infarction in men (73, 74). The protective mechanism of lycopene against cardiovascular disease seems to be associated more with its hypolipidemic activity than its antioxidant capacity. In fact lycopene-enriched LDLs were not protected from oxidation (70). Thus effects other than the antioxidant may explain the negative association between lycopene and cardiovascular risk. Additional studies have shown that lycopene has a hypolipidemic effect as a result of suppression of cholesterol synthesis and stimulation of its removal, resulting in a significant reduction in plasma cholesterol without significant changes in high-density lipoprotein (HDL) cholesterol concentrations (75).

In addition to antioxidant vitamins, polyphenols, a class of phytochemicals widely represented in fruit, plant-derived beverages, and red wine are characterized by an antioxidant activity, which prevents LDL oxidation (76-79). Once ingested, polyphenols are partly incorporated into lipoproteins and are partly circulating

in soluble form in plasma. Administration of polyphenol-enriched nutrients reduces systemic oxidative stress, improves coronary circulation, and reduces endothelial dysfunction and atherosclerosis (80-82). It now appears clear that, in addition to being an antioxidant, polyphenols exert a spectrum of other actions which could delay atherosclerosis onset and progression (83). When administered orally, grape flavonoids inhibit platelet aggregation, monocyte adhesion to the endothelium, thrombus formation, and nuclear factor kappa beta activation (84-87). They also stimulate vasodilation (88) and increase plasma HDL cholesterol (82). The beneficial effect of polyphenols on plasma lipids seems to be mediated by the activation of the enzyme adenosine monophosphate-activated protein kinase (AMPK), a master regulator of intermediate metabolism (89). AMPK is a widespread enzyme in tissues, which is activated by an increase in the ADP/ATP ratio. AMPK activation results in inhibition of protein, free fatty acids and cholesterol synthesis, while enhancing glucose transport within cells and glycolysis and stimulating  $\beta$ -oxidation of fatty acids (90). In the vasculature, activation of AMPK increases the production of nitric oxide and the oxidation of free fatty acids, and reduces endothelial cell apoptosis and vascular smooth muscle cell proliferation (91-94). Administration of resveratrol, a polyphenolic compound of red wine inhibits accelerated atherosclerosis in animal models via AMPK activation (89).

### **Nutrition and endothelial function**

Nitric oxide plays a key role in vascular biology for its well-recognized antiatherosclerotic properties (95). In 1980, Furchgott and Zawadzki demonstrated that acetylcholine-induced vasodilation depended upon its presence in the endothelium and that this effect was mediated by a labile molecule, known as endothelium-derived-hyperpolarizing-factor and later identified as nitric oxide (NO) (96). Biochemically, nitric oxide is produced in the endothelium from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS) (97). Three isoforms of NOS exist (98): first, neuronal NOS (nNOS) is expressed in brain and in skeletal muscle. Second, inducible NOS (iNOS), which is expressed in macrophages and endothelial cells, is a cytosolic, calcium-independent enzyme, activated by cytokines. It produces large amounts of NO in a short time and is involved in the inflammatory burst in leukocytes (99). And, third, endothelial NOS (eNOS) (100) is a calcium-calmodulin dependent enzyme, which is active constitutively. In physiologic conditions, it releases small amounts of NO continuously; however, it is activated

also in a receptor-dependent fashion by physiologic stimuli such as shear stress, estrogens, insulin, and some adipocytokines. NO is characterized by a short half-life. Once it is released in the endothelium it diffuses to the vascular lumen and to the underlying vascular smooth muscle cells, where it activates the soluble guanylate cyclase. This enzyme converts the guanosine triphosphate in the second messenger cyclic guanosine monophosphate (cGMP), which is responsible for relaxation of vascular smooth muscle cells. cGMP is also involved in other effects of NO, such as inhibition of platelet adhesion, leukocyte aggregation, and smooth muscle cell proliferation (95). In addition, recent evidence shows additional antiatherosclerotic effects of NO, which are cGMP independent: i.e., inhibition of endothelial cell apoptosis and of the nuclear factor kappa beta, a pivotal enzyme in the proinflammatory response (101). Thus eNOS-derived NO is a central molecule for a healthy vasculature, and loss of NO bioavailability is an important factor in the pathogenesis of endothelial dysfunction and atherosclerosis. To generate NO, eNOS converts L-arginine into L-citrulline. In this reaction, the free radical superoxide anion is also formed in physiologic conditions. For the optimal functionality of the enzyme, appropriate amounts of the cofactors are required, in particular of tetrahydrobiopterin derived from folic acid, which in addition modulates homocysteine production. In the absence of tetrahydrobiopterin, the enzyme is uncoupled and produces large amounts of superoxide anion in front of small quantities of NO (102). Deficiency of tetrahydrobiopterin has been demonstrated in human atherosclerosis (103), and supplementation with folic acid results in decreased plasma homocysteine levels, improved endothelial function, and reduced formation of superoxide anion in human atherosclerosis (104, 105). Interestingly, the positive effects of folic acid acute supplementation are already detectable in the presence of small amounts (5 mg) of the precursor (106). This observation may explain the discrepancy between epidemiologic data suggesting beneficial effects of folic acid supplementation on cardiovascular disease and clinical trials showing little benefit from folic acid supplementation on cardiovascular outcome in countries where mandatory cereal folate fortification exists (107). However, analysis of subgroups in the Heart Outcomes Prevention Evaluation 2 (HOPE-2) study has shown a trend toward reduced relative risk of new events in subjects who were not receiving folate fortification. In addition, folic acid supplementation significantly reduced the risk of stroke in this group (108). Thus, folic acid supplementation may represent an inexpensive and safe nutritional approach for the prevention and treatment of cardiovascular disease

in selected populations (109).

Another important precursor of nitric oxide is the substrate of the enzyme, arginine. Arginine administration results in vasodilation and restored endothelial function in atherosclerosis and hypercholesterolemia, which is probably the result of the trapping effect of arginine on asymmetric dimethylarginine, a NOS inhibitor which is increased in aging, diabetes, atherosclerosis, and chronic renal failure (110). Therefore, arginine supplementation has been proposed as a nutritional intervention for cardiovascular disease. However, the recently published Vascular Interaction with Age in Myocardial Infarction (VINTAGE-MI) study has demonstrated that arginine supplementation 9 g/day for 6 months in subjects 60 years of age with previous myocardial infarction did not result in improved ejection fraction and lower vascular stiffness. In addition, cardiovascular mortality increased significantly (111). These findings suggest that caution is needed in the administration of this amino acid in elderly subjects with a previous history of cardiovascular disease.

#### **Nutrition and endothelial function: implications for clinical practice**

Nutritional counseling is very important in the primary and secondary prevention of cardiovascular disease. Based on current clinical guidelines, patients should first of all be encouraged to assume diets rich in fruit and vegetables, and it should be emphasized that the protective effects of these diets occur at dietary levels of nutrient antioxidants in the 2-3 times the recommended dietary intake (RDI) range. Routine vitamin A, C, and E supplementation is currently not recommended, based on the results of randomized clinical trials, which have been biased by confounding factors: i.e., different vitamin sources (natural vs. synthetic vitamin E) and different doses and drug combinations (i.e., addition of statins). Dietary antioxidant (AOX) supplements may be indicated only in patients whose diet does not provide the recommended dietary intake for specific antioxidant vitamins, with selective exceptions (e.g. beta carotene in smokers).

In addition, whole-grain, high-fiber foods should be included. As mentioned above, cereal folate fortification is sufficient to restore endothelial function and prevent superoxide production from human vessels. Folic acid supplementation is currently not routinely recommended for the primary and secondary prevention of cardiovascular disease. However, it is recommended in selected patient groups: e.g. pregnant women, and patients with documented deficiency of folic acid, secondary anemia, and hyperhomocystinemia associated

with mild to moderate cognitive impairment. The intake of sodium, saturated fat, cholesterol, and *trans*-fatty acids should be limited; in contrast, the consumption of omega-3 fatty acids in the form of fish or in capsule form for risk reduction is recommended both in primary and secondary prevention strategies. Omega-3 fatty acids (approximately 850 to 1,000 mg of EPA and DHA) should be considered in patients with previous acute myocardial infarction and/or evidence of coronary heart disease, and higher doses (2 to 4 g) may be used in patients with high triglyceride levels.

#### **CONCLUSIONS**

Inflammation and oxidative stress are a feature of atherosclerosis, through negative effects on NO bioavailability and via interactions with numerous inflammatory-driven signaling pathways. A number of different strategies are available to prevent and delay cardiovascular disease onset and progression, either by preserving nitric oxide bioavailability, blunting inflammation, or lowering oxidative stress.

The effects of AOX vitamins have been extensively addressed in clinical trials. The available evidence is inconclusive due to confounding variables on target outcomes. Therefore, so far there is insufficient evidence to recommend routine AOX vitamin supplementation in clinical practice in the general population. Patients should instead be encouraged to assume a diet rich in fruit and vegetables, as AOX vitamins are abundantly represented in these diets.

The effects of new emerging antioxidants on cardiovascular disease have been less characterized in epidemiologic studies. In addition, new effects of polyphenolic compounds are emerging, possibly influencing the clinical course of atherosclerosis. Optimization of NO bioavailability through folic acid seems a valid approach for vascular disease. Finally, attenuation of vascular inflammation through omega-3 polyunsaturated fatty acids represents a winning nutritional strategy in the treatment of cardiovascular disease.

**Financial support:** none.

**Conflict of interest:** none declared.

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Received: April 14, 2008

Accepted: June 3, 2009